Dysregulation of glucocorticoid metabolism in murine obesity

Dysregulation of glucocorticoid metabolism in murine obesity: comparable effects of leptin resistance and deficiency

Dawn E W Livingstone, Sarah L Grassick, Gillian L Currie, Brian R Walker and **Ruth Andrew**

Endocrinology Unit, Queen's Medical Research Institute, Centre for Cardiovascular Science, University of Edinburgh, 47, Little France Crescent, Edinburgh EH16 4TJ, UK

(Correspondence should be addressed to R Andrew; Email: ruth.andrew@ed.ac.uk)

Abstract

In obese humans, metabolism of glucocorticoids by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and A-ring reduction (by 5α- and 5β-reductases) is dysregulated in a tissue specific manner. These changes have been recapitulated in leptin resistant obese Zucker rats but were not observed in high-fat fed Wistar rats. Recent data from mouse models suggest that such discrepancies may reflect differences in leptin signalling. We therefore compared glucocorticoid metabolism in murine models of leptin deficiency and resistance. Male *ob/ob* and *db/db* mice and their respective littermate controls ($n=10$–12/group) were studied at the age of 12 weeks. Enzyme activities and mRNA expression were quantified in snap-frozen tissues. The patterns of altered pathways of steroid metabolism in obesity were similar in *ob/ob* and *db/db* mice. In liver, 5β-reductase activity and mRNA were increased and 11β-HSD1 decreased in obese mice, whereas 5α-reductase 1 (5αR1) mRNA was not altered. In visceral adipose depots, 5β-reductase was not expressed, 11β-HSD1 activity was increased and 5αR1 mRNA was not altered in obesity. By contrast, in subcutaneous adipose tissue 11β-HSD1 and 5αR1 mRNA were decreased. Systematic differences were not found between *ob/ob* and *db/db* murine models of obesity, suggesting that variations in leptin signalling through the short splice variant of the Ob receptor do not contribute to dysregulation of glucocorticoid metabolism.

Journal of Endocrinology (2009) **201,** 211–218

Introduction

Tissue-specific dysregulation of the glucocorticoid-generating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in rodent (Livingstone *et al.* 2000, Liu *et al.* 2003) and human obesity (Bujalska *et al.* 1997, Fraser *et al.* 1999, Rask *et al.* 2001) and the effects of 11β-HSD1 deficiency (Morton *et al.* 2004) or inhibition (Alberts *et al.* 2002, Hermanowski-Vosatka *et al.* 2005) to protect against obesity-associated metabolic dysfunction, has supported the hypothesis that variations in glucocorticoid metabolism within target tissues play an important role in the pathophysiology of Metabolic Syndrome.

In addition to 11β-HSD1, glucocorticoids are metabolised by several other enzymes (Fig. 1). In liver, glucocorticoids are inactivated by A-ring reductases (5α- and 5β-reductases and 3α-HSD) and regenerated by 11β-HSD1 (Andrew & Walker 2002). The pattern of metabolism in adipose tissue is similar, with 5α-reductase 1 (5αR1), 3α-HSD and 11β-HSD1 being expressed (Barat *et al.* 2007, Wake *et al.* 2007*b*). A-ring reductases are also dysregulated in obesity. In human obesity, whole body A-ring reduction is enhanced, as judged by urinary steroid excretion, increasing peripheral clearance of cortisol and (Andrew *et al.* 1998, Tomlinson *et al.* 2008) potentially inducing compensatory activation of the hypothalamic–pituitary–adrenal axis (Andrew *et al.* 1998, Rask *et al.* 2001, 2002). In leptin-resistant obese Zucker rats increased urinary 5α-reduced metabolites can be accounted for by up-regulation of expression and activities of hepatic A-ring reductases (Livingstone *et al.* 2005). 5αR1 is also expressed in adipose tissue but is not dysregulated in subcutaneous (s.c.) adipose in obese humans or rats (Barat *et al.* 2007, Wake *et al.* 2007*b*).

In studies of 11β-HSD1, up-regulation of enzyme expression in adipose tissue and down-regulation in liver has not been a universal finding in obesity. For example, in diet-induced obesity in mice and rats adipose 11β-HSD1 is down-regulated (Morton *et al.* 2004, Drake *et al.* 2005). Furthermore, leptin resistant and deficient mice have been shown by Liu *et al.* to have divergent changes in hepatic 11β-HSD1 activity, with expression and activity being decreased in leptin deficient *ob/ob* mice (Liu *et al.* 2003) but paradoxically increased in leptin resistant *db/db* mice (Liu *et al.* 2005). These differences may reflect the distinctions between the defects in leptin signalling in these models.

Leptin signals through several splice variants of the leptin receptor (Ob-R; Lee *et al.* 1996). The long-form of the receptor (Ob-Rb) has an intracellular domain crucial to its signalling properties via Stat3, and is predominantly expressed in the hypothalamus, where it controls appetite regulation; this

DOI: 10.1677/JOE-09-0003
0022–0795/09/0201–211
Online version via http://www.endocrinology-journals.org

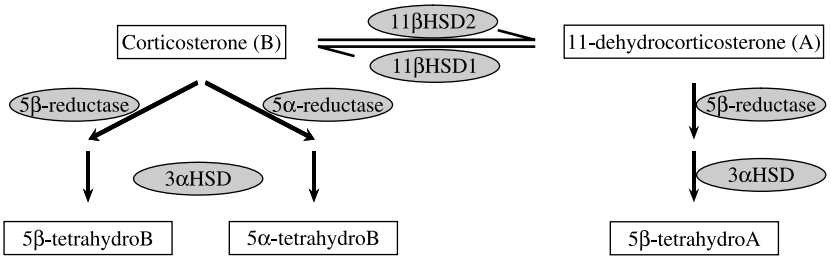

Figure 1 Glucocorticoid metabolic pathways. A is 11-dehydrocorticosterone; B is corticosterone and HSD is hydroxysteroid dehydrogenase.

cytosolic region is truncated in the *db/db* mouse (Lee *et al.* 1996, Sahai *et al.* 2004). A short form of the receptor lacking the intracellular domain (Ob-Ra) is expressed in the liver (Hoggard *et al.* 1997), where it can activate the inositol trisphosphate kinase cascade, thus potentially modulating insulin signalling pathways (Cohen *et al.* 1996, Zhao *et al.* 2000). Stimulation of Ob-Ra may occur in *db/db* mice, but not in *ob/ob* mice that lack circulating leptin. Indeed, Ob-Ra activation may be critical in the development of hepatic insulin resistance and non-alcoholic steatohepatitis (Sahai *et al.* 2004). It is possible that activation of Ob-Ra may be responsible for leptin-induced up-regulation of 11β-HSD1 expression in hepatocytes (which lack Ob-Rb; Liu *et al.* 2005), and this mechanism may mediate up-regulation of liver 11β-HSD1 in *db/db* mice. This capacity for residual leptin signalling in *db/db* mice is not predicted in Zucker obese rats, since the *fa* mutation in the leptin receptor affects the extracellular domains of both Ob-Ra and b (Chua *et al.* 1996, Da Silva *et al.* 1998). We therefore hypothesised that deficient leptin signalling underlies dysregulation of hepatic glucocorticoid metabolism by 11β-HSD1 and A-ring reductases.

The aims of the present study were to explore glucocorticoid metabolism – not only by 11β-HSD1 but also by A-ring reductases – in murine models of obesity and, by comparing findings in *ob/ob* and *db/db* mice, to dissect the potential role of Ob-Ra in mediating dysregulation of hepatic steroid metabolism in obesity.

Materials and Methods

Materials

All chemicals were obtained from Sigma unless otherwise stated. Solvents were glass distilled HPLC grade from Fisher Scientific (Loughborough, UK). Steroid standards were obtained from Steraloids (Newport, RI, USA). Radiolabelled-steroids were from GE Healthcare (Buckinghamshire, UK).

Animals

Male obese ($Lepr^{DB}/Lepr^{DB}$ (*db/db*) and Lep^{ob}/Lep^{ob} (*ob/ob*)) mice and their respective lean heterozygote or wild-type littermates (*Db/?* and *Ob/?*; C57BL background; Harlan, Bicester, UK) were characterised by phenotype, maintained under controlled conditions of light (on 0700–1900 h) and temperature (21 °C), and allowed free access to standard chow (Special Diet Services, Witham, UK) and drinking water. At 12 weeks of age, they were decapitated at 0800–1100 h and tissues dissected and snap frozen on dry ice. All animal experiments were carried out under UK Home Office guidelines.

Biochemical assays

Glucose and insulin were measured by hexokinase (Thermo Electron, Melbourne, Australia) and ELISA (Crystal Chem Inc., Downers Grove, IL, USA) respectively. Hepatic triglycerides were measured spectrophotometrically (Microgenics, Passau, Germany) as previously reported (Raubenheimer *et al.* 2006). Corticosterone was quantified in plasma by in-house RIA (Holmes *et al.* 1995).

11β-HSD1 activity assay

11β-HSD1 is a reductase *in vivo*, converting inactive 11-dehydrocorticosterone to corticosterone. However, *in vitro* dehydrogenase activity predominates and measurements of reductase activity are confounded by competition with other enzymes. Therefore, to estimate 11β-HSD1 protein, we measured enzyme activity as conversion of corticosterone to 11-dehydrocorticosterone in the presence of an excess of cofactor $NADP^+$. Aliquots of tissues were homogenised in Krebs Ringer buffer as previously described (Livingstone *et al.* 2000), and protein concentrations determined colorimetrically using a Bradford kit (Bio-Rad). Standardised amounts of protein for each tissue were incubated in duplicate at 37 °C in Krebs Ringer buffer containing 0·2% glucose, $NADP^+$ (2 mmol/l), $[^3H]_4$-corticosterone (10 nmol/l) and unlabelled corticosterone (1·99 μmol/l). Protein concentrations and incubation times were optimised for each tissue to ensure first order kinetics (liver, 25 μg/ml per h; adipose tissue, 100–200 μg/ml per h). After incubation, steroids were extracted with ethyl acetate, the organic phase evaporated under nitrogen and extracts re-solubilised in mobile phase (water:acetonitrile:methanol; 60:15:25, 1·5 ml/min). Steroids were separated by HPLC using a C18

reverse phase Symmetry column (4·6 mm, 15 cm, 5 μm; Waters, Elstree, UK) at 35 °C and quantified by on-line liquid scintillation counting.

Owing to the paucity of intra-abdominal adipose tissue in lean mice, omental adipose tissue was used to quantify enzyme activity, whereas mesenteric adipose was used to quantify transcript abundance.

5β-Reductase activity assay

Hepatic 5β-reductase (5βR) activity was assessed by the conversion of $[^3H]_4$-corticosterone to $[^3H]_4$-5β-tetrahydrocorticosterone in hepatic cytosol (Livingstone *et al.* 2005). Enzyme velocity was measured by incubating cytosol in duplicate at 37 °C, in sodium phosphate buffer (40 mmol/l Na_2PO_4, 320 mmol/l sucrose, 1 mmol/l dithiothreitol, pH 7·5) containing NADPH (1 mmol/l), glucose-6-phosphate (5 mmol/l M), glucose-6-phosphate dehydrogenase (1 unit/ml), $[^3H]_4$-corticosterone (10 nmol/l) and unlabelled corticosterone (1·99 μmol/l; Livingstone *et al.* 2005). Protein concentration and incubation period (0·5 mg/ml for 24 h) were optimised to ensure first order kinetics. Steroids were extracted with ethyl acetate, the organic phase was evaporated under nitrogen and extracts re-solubilised in mobile phase and analysed by HPLC as above.

Quantification of mRNA by real-time quantitative PCR

Total RNA was extracted from snap-frozen tissue samples, and 500 ng reverse transcribed into cDNA with random primers using the QuantiTect DNase/reverse transcription kit (Qiagen Ltd). cDNA (equivalent to 10 ng total RNA) was incubated in triplicate with 1× gene specific assay mix (Applied Biosystems, Warrington, UK) in 1× LightCycler480 Probes mastermix (Roche Diagnostics Ltd). PCR cycling and detection of fluorescent signal was carried out using a Roche LightCycler480. A standard curve was constructed for each primer probe set using a serial dilution of cDNA pooled from all samples. For liver and adipose, results were corrected for 18S and cyclophilin A RNA respectively, which were not different between groups. Assays used were: 11β-HSD1, Mm00476182_m1; 5αR1, Mm00614213_m1; 5βR, Mm00520266_m1; 18S, Hs99999 901_s1 and Cyclophilin A, Mm02342430_g1.

Statistical analysis

Data are mean ± S.E.M. and groups ($n = 10$–12 unless otherwise stated) were compared by Student's *t*-test.

Results

Both *db/db* mice and *ob/ob* mice were heavier than their respective control groups at the time of cull (Table 1), and had increased liver weight. Both *db/db* and *ob/ob* mice had higher circulating glucose, insulin and corticosterone and hepatic triglycerides than lean controls.

Hepatic glucocorticoid metabolism

Hepatic 11β-HSD1 activity was lower in both *db/db* and *ob/ob* mice compared with lean controls (Fig. 2A and B), although mRNA for 11β-HSD1 was not different in either model (Fig. 2E and F). 5βR activity and transcript abundance were higher in both *db/db* and *ob/ob* mice compared with their controls (Fig. 2C–F). There was no difference in abundance of 5αR1 mRNA between lean and obese animals of either strain (Fig. 2E and F). Activity of 5αR1 was not measured due to instability of the protein (Eicheler *et al.* 1995).

Glucocorticoid metabolism in adipose tissue

In *db/db* mice, activity of 11β-HSD1 was higher in retroperitoneal and omental adipose but lower in s.c. and epididymal adipose compared with controls (Fig. 3A). In *ob/ob* mice, 11β-HSD1 activity was higher in epididymal, retro-peritoneal and omental adipose but lower in s.c. adipose tissue compared with controls (Fig. 3B). Expression of 11β-HSD1 mRNA followed a similar pattern, in the main (Fig. 3C and D), although dysregulation of 11β-HSD1 mRNA was not observed in epididymal adipose tissue. Note that in mesenteric adipose tissue, limited amounts of tissue in lean mice resulted in analysis of only $n = 6$ samples, and hence borderline statistical significance for the up-regulation of abundance of 11β-HSD1 mRNA in *db/db* mice.

Abundance of mRNA for 5αR1 was lower in s.c. adipose tissue from obese mice of both strains compared with their respective controls, but was not altered in mesenteric,

Table 1 Body and liver weights and plasma biochemistry of mice

	***Db/?* control**	***db/db***	***Ob/?* control**	***ob/ob***
Weight at cull (g)	25·0 ± 0·54	34·5 ± 0·92*	25·7 ± 0·46	42·1 ± 0·55†
Liver (g)	1·31 ± 0·02	2·14 ± 0·14*	1·41 ± 0·08	3·79 ± 0·04†
Glucose (mg/dl)	223 ± 14	550 ± 68*	207 ± 109	367 ± 42†
Insulin (pg/ml)	1·2 ± 0·2	5·5 ± 0·9*	0·5 ± 0·1	>12·8†
Liver TAG (μmol/g)	34·7 ± 3·5	728 ± 178*	36·3 ± 5·3	2287 ± 130†
Corticosterone (nM)	7·2 ± 1·4	60·3 ± 19·5**	17·9 ± 4·7	77·4 ± 16·0†

Data are mean ± S.E.M., compared by Student's *t*-test. *$P < 0·005$, **$P < 0·01$ for *db/db* mice versus *Db/?* control. †$P < 0·005$ versus *ob/ob* mice versus *Ob/?* control. $N = 10$–13/group. NB insulin concentrations in all *ob/ob* mice exceeded the maximum point of the assay. TAG, triglycerides.

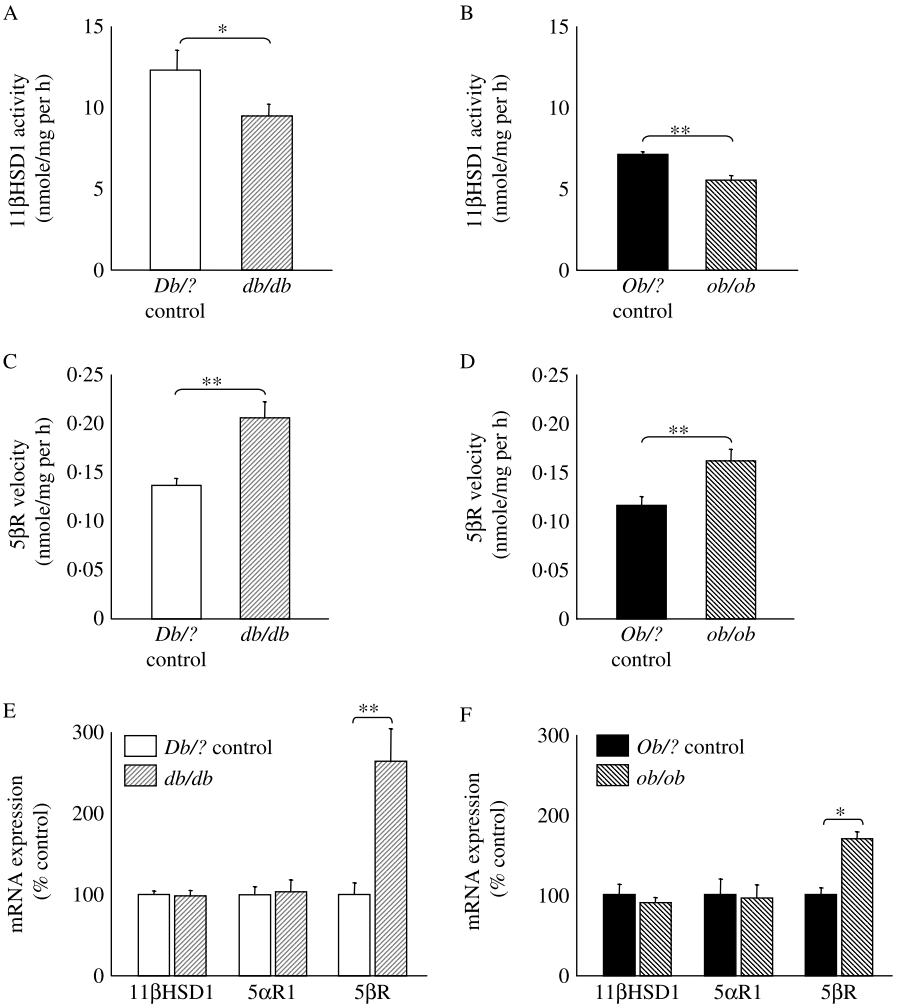

Figure 2 Hepatic glucocorticoid metabolism. 11β-HSD1 activity measured as velocity of formation of product following incubation of $[^3H]_4$-corticosterone with hepatic homogenate from (A) *Db/?* control (open) or *db/db* mice (light striped); (B) *Ob/?* control (black) or *ob/ob* mice (dark striped). 5β-Reductase activity measured as velocity of formation of product following incubation of $[^3H]_4$-corticosterone with hepatic cytosol from (C) *Db/?* control or *db/db* mice; (D) *Ob/?* control or *ob/ob* mice. Abundance of mRNAs for hepatic enzymes measured by real-time PCR (corrected for 18S as a housekeeping gene and presented as a percentage of respective control group) in (E) *Db/?* control or *db/db* mice; (F) *Ob/?* control mice or *ob/ob* mice. Data are mean±S.E.M.; $n=10$–12/group; $^*P<0{\cdot}05$; $^{**}P<0{\cdot}01$.

epididymal or retro-peritoneal adipose tissue in either obese strain (Fig. 3E and F).

Neither 5β- nor 5α-reductase 2 mRNAs were detected in adipose tissue.

Discussion

These studies demonstrate that mice with genetic obesity due to either defective leptin secretion (*ob/ob*) or sensitivity (*db/db*) have similar alterations in 11β-HSD1 and 5βR as Zucker obese rats (Livingstone *et al.* 2005). This includes down-regulation of 11β-HSD1 in liver and up-regulation in visceral adipose tissue depots, although in obese mice 11β-HSD1 was lower in s.c. adipose depots. By contrast with Zucker rats (Livingstone *et al.* 2005), however, 5αR1 expression was not increased in liver of obese mice and was decreased in s.c. adipose tissue. Strikingly, we did not find systematic differences between glucocorticoid metabolism in leptin deficient *ob/ob* mice and leptin-resistant *db/db* mice. This contrasts with the previous reports suggesting up-regulation of 11β-HSD1 in the liver of *db/db* mice (Liu *et al.* 2005, Nakano *et al.* 2007), and suggests that enhanced signalling through the short Ob-Ra splice variant does not contribute to

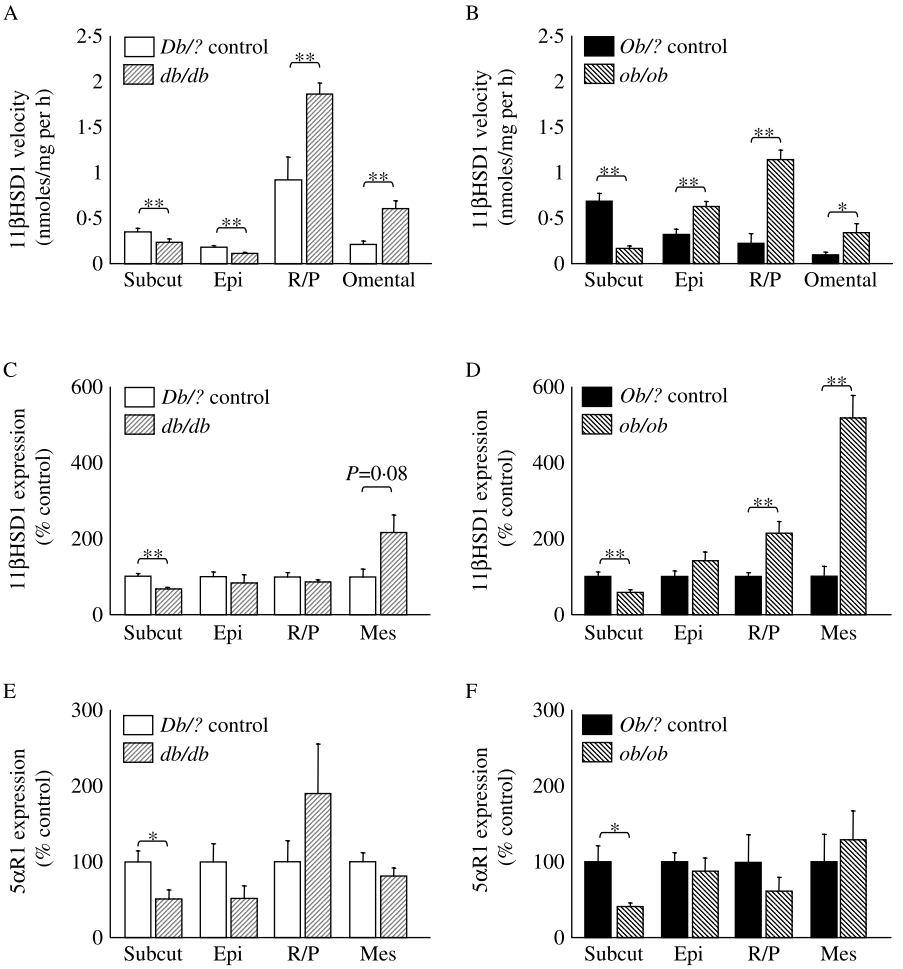

Figure 3 Glucocorticoid metabolism in adipose tissues. 11β-HSD1 activity measured as velocity of formation of product following incubation of $[^3H]_4$-corticosterone with homogenates of adipose tissues from (A) *Db/?* control (open) or *db/db* mice (light striped); (B) *Ob/?* control (black) or *ob/ob* mice (dark striped). Abundance of mRNA for 11β-HSD1 in adipose tissue depots measured by real-time PCR (corrected for cyclophilin A as a housekeeping gene and presented as a percentage of respective control group) in (C) *Db/?* control or *db/db* mice; (D) *Ob/?* control or *ob/ob* mice. Abundance of mRNAs for 5α-R1 in adipose beds measured by real-time PCR (corrected for cyclophilin A as a housekeeping gene and presented as a percentage of respective control group) in (E) *Db/?* control or *db/db* mice; (F) *Ob/?* control or *ob/ob* mice. Subcut is s.c. adipose, Epi is epididymal, R/P is retro-peritoneal and Mes is mesenteric. Data are mean ± S.E.M.; $n=6$–12/group; $^*P<0{\cdot}05$; $^{**}P<0{\cdot}01$.

the regulation of hepatic glucocorticoid metabolism *in vivo*. Previously observed effects of leptin administration *in vivo* to reverse changes in 11β-HSD1 in *ob/ob* mice (Liu *et al*. 2003) may have been mediated indirectly through weight loss and reversal of the metabolic phenotype, which inevitably follow leptin replacement.

The pattern of dysregulation of glucocorticoid metabolism in obese rodents differs in some respects from that in obese humans. In human adipose tissue, up-regulation of s.c. 11β-HSD1 is widely reported but 5αR1 is not altered (Wake *et al*. 2007*b*) and alterations in visceral adipose 11β-HSD1 are inconsistent (Walker & Andrew 2006). In human liver, down-regulation of 11β-HSD1 and up-regulation of both 5αR1 and 5βR activity have been reported consistently (Andrew *et al*. 1998, Fraser *et al*. 1999, Rask *et al*. 2001, Tomlinson *et al*. 2008), and some of these hepatic changes are paralleled here in mice and in our studies of obese Zucker rats (Livingstone *et al*. 2000, 2005, Barat *et al*. 2007).

This species specificity provides a potential opportunity to dissect mechanisms determining dysregulation of glucocorticoid metabolism in humans using comparative studies in rodents. However, given limited knowledge of regulation of expression of A-ring reductases, the mechanism of altered A-ring reductase activity in obesity remains uncertain. 5βR is

transiently up-regulated in rats fed a high-fat diet (Drake *et al.* 2005), and in humans is selectively up-regulated in insulin resistance associated with fatty liver (Westerbacka *et al.* 2003). The murine models reported here had markedly fatty liver, moreso than that induced with diet-induced obesity. However, the severity of steatosis was more marked in the *ob/ob* mice, whereas the activity of 5β-reductase was increased to a greater extent in *db/db* mice. The *db/db* mice demonstrated partial insulin deficiency, and progression towards hyperglycaemia, whereas the *ob/ob* mice maintained near to normal glucose concentrations, albeit with higher insulin. This perhaps implicates the elevated insulin concentrations themselves in the dysregulation of steroid metabolism. The other potent regulators of 5βR identified to date are androgens, which imprint permanent down-regulation of 5βR in liver following *in utero* exposure (Einarsson & Gustafsson 1973, Gustafsson & Stenberg 1974, Jansson *et al.* 1985). In addition, withdrawal of androgens increases 5βR (Barat *et al.* 2007). Hence, the observed up-regulation may reflect the characteristic lowering of circulating androgens in obesity (Whitaker *et al.* 1983, Zumoff *et al.* 1990).

Regarding the regulation of 5αR1, these data suggest that the mechanism of dysregulation in human obesity is context and/or species-specific and does not operate in *ob/ob* or *db/db* mice. A caveat, however, is that protein levels or activity of 5αR1 might vary in the absence of changes in mRNA, but this cannot be readily tested given the instability of the hepatic 5αR1 protein *ex vivo* (Eicheler *et al.* 1995). Previous reports in both humans and rats support the notion that up-regulation of 5αR1 is secondary to the development of insulin resistance/hyperinsulinaemia and is reversible on treatment (Tsilchorozidou *et al.* 2003, Livingstone *et al.* 2005, Tomlinson *et al.* 2008). IGF-1 has been suggested as the principle candidate for dysregulation of hepatic 5αR1 (Horton *et al.* 1993). However, both mouse strains studied exhibit profound insulin resistance and therefore this explanation may be overly simplistic. Studies of 5αRs in mouse reproductive physiology have highlighted a possible redundancy between the two isozymes compared with other species (Mahendroo *et al.* 1996, 2001), but this is unlikely to explain the differences in 5αR1 dysregulation in obese mice, since the expression of 5αR2 was not detected in either liver or adipose tissue.

The few reports to date examining 5αR1 in adipose tissue in humans and Zucker rats suggest that the abundance of transcript is not altered by obesity in s.c. depots (Barat *et al.* 2007, Wake *et al.* 2007*b*). However, in both *ob/ob* and *db/db* mice, 5αR1 mRNA was down-regulated selectively in s.c. adipose, again highlighting the differences in regulation in this enzyme between species. In contrast to Zucker rats, in which 5αR1 expression was increased in omental adipose tissue (Barat *et al.* 2007), changes in mRNA expression were not observed in the murine mesenteric depot, although the greater omental depot was not studied as a direct comparison due to a paucity of tissue in lean mice.

Regulation of 11β-HSD1 transcription has been studied extensively but the basis for tissues-specific dysregulation in obesity remains elusive. Species differences in regulation of 11β-HSD1 have been demonstrated, most recently in relation to PPAR agonists (Hermanowski-Vosatka *et al.* 2000, Wake *et al.* 2007*a*). Elevated circulating glucocorticoid levels, that are much more striking in rodent than in human obesity, may contribute since 11β-HSD1 is a glucocorticoid-responsive gene (Low *et al.* 1994, Jamieson *et al.* 1995, Voice *et al.* 1996). The striking observation in murine obesity in the present data is the down-regulation of 11β-HSD1 in s.c. adipose tissue. This has also been observed in diet-induced and in polygenic obesity in mice (Morton *et al.* 2004, 2005). However in humans, inhibition of 11β-HSD1 in s.c. adipose tissue has become an attractive target for restricting glucocorticoid action, with most groups agreeing, that in humans, there is up-regulation of the enzyme in this depot (Paulmyer-Lacroix *et al.* 2002, Lindsay *et al.* 2003, Wake *et al.* 2003). Of interest is the observation that changes in 11β-HSD1 activity in both obese models are more marked than changes in mRNA, indeed in epididymal fat mRNA was not altered. This discrepancy has been reported by ourselves (Morton *et al.* 2004) and others (Bujalska *et al.* 2005, Jang *et al.* 2006) previously. The relationship between activity and abundance of transcript appears most robust in s.c. adipose in humans (Wake *et al.* 2003, Goedecke *et al.* 2006); discrepancies existing at other sites and observed here may reflect an additional level of control of 11β-HSD1 protein by post-translational modification, e.g. glycosylation (Opperman *et al.* 1995). Another source of variation between species and depots is the mixture of cell types. 11β-HSD1 is expressed in macrophages as well as adipocytes (Gilmour *et al.* 2006). There is emerging evidence that some depots, and some animal models are more susceptible to macrophage infiltration in the adipose tissue in obesity (Surmi & Hasty 2008).

In conclusion, murine obesity is characterised by some but not all of the changes in steroid metabolism that are observed in human obesity. The consequences of disrupted glucocorticoid metabolism in rodents may differ from those in humans, since rodents also exhibit substantially elevated circulating concentrations of corticosterone, contrasting with low to normal circulating cortisol in human obesity (Phillips *et al.* 2000). Nevertheless, mice may provide useful models in which to investigate dysregulation of 5βR and 11β-HSD1 but not 5αR1 in liver. None of these changes differ substantially in mice with or without leptin signalling through Ob-Ra. The pattern of dysregulation of metabolism in adipose tissue is, however, subtly different between species, offering the possibility that further comparative biology studies may elucidate relevant mechanisms.

Declaration of interest

D E W L, S L G, G L C and R A have no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported. Within the past 2 years, B R W has consulted for Astra-Zeneca, Dainippon Sumitomo, Merck, Johnson & Johnson, Incyte, Ipsen, Roche, Vitae, Wyeth, Zydus

Research Centre, received lecture fees from Abbott and Bristol Myers Squibb, and received research funding from Wyeth. B R W is an inventor on relevant patents held by University of Edinburgh.

Funding

This work was supported by the Wellcome Trust (060707 and VS/06/UED/A8).

Author contribution statement

D E W L, S L G, G L C contributed to the execution and analysis of the studies. D E W L, B R W and R A contributed to study design, data analysis and interpretation and preparation of the manuscript.

Acknowledgements

We are grateful to Mrs Carolynn Cairns and Mrs Rachel McDonnell for their excellent technical support.

References

Alberts P, Engblom L, Edling N, Forsgren M, Klingstrom G, Larsson C, Rönquist-Nii Y, Öhman B & Abrahmsén L 2002 Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. *Diabetologia* **45** 1528–1532.

Andrew R & Walker BR 2002 Glucocorticoid metabolism in health and disease. *Recent Research Developments in Endocrinology* **3** 425–449.

Andrew R, Phillips DIW & Walker BR 1998 Obesity and gender influence cortisol secretion and metabolism in man. *Journal of Clinical Endocrinology and Metabolism* **83** 1806–1809.

Barat P, Livingstone DEW, Elferink C, MacDonnell R, Walker BR & Andrew R 2007 Effects of gonadectomy on glucocorticoid metabolism in obese Zucker rats. *Endocrinology* **148** 4836–4843.

Bujalska IJ, Kumar S & Stewart PM 1997 Does central obesity reflect 'Cushing's disease of the omentum'? *Lancet* **349** 1210–1213.

Bujalska IJ, Draper N, Michailidou Z, Tomlinson JW, White PC, Chapman KE, Walker EA & Stewart PM 2005 Hexose-6-phosphate dehydrogenase confers oxo-reductase activity upon 11β-hydroxysteroid dehydrogenase type 1. *Journal of Molecular Endocrinology* **34** 675–684.

Chua SC, Chung WK, Wu-Peng XS, Zhang Y, Liu S-M, Tartaglia L & Leibel RL 1996 Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* **271** 994–996.

Cohen B, Novick D & Rubinstein M 1996 Modulation of insulin activities by leptin. *Science* **274** 1185–1188.

Drake AJ, Livingstone DEW, Morton NM, Andrew R, Seckl JR & Walker BR 2005 Reduced adipose glucocorticoid reactivation and increased hepatic glucocorticoid clearance as an early adaptation to high fat feeding in rats. *Endocrinology* **146** 913–919.

Eicheler W, Seitz J, Steinhoff M, Forssmann WG, Adermann K & Aumuller G 1995 Distribution of rat hepatic steroid 5alpha-reductase 1 as shown by immunohistochemistry. *Experimental and Clinical Endocrinology and Diabetes* **103** 105–112.

Einarsson K & Gustafsson J-A 1973 Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. *Journal of Biological Chemistry* **248** 4987–4997.

Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E & Connell JMC 1999 Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *American Journal of Physiology. Endocrinology and Metabolism* **33** 1364–1368.

Gilmour JS, Coutinho AE, Cailhier J-F, Man TY, Clay M, Thomas G, Harris HG, Mullins JJ, Seckl JR, Savill JS *et al.* 2006 Local amplification of glucocorticoids by 11beta-hydroxysteroid dehydrogenase type 1 promotes macrophage phagocytosis of apoptotic leukocytes. *Journal of Immunology* **176** 7605–7611.

Goedecke JH, Wake DJ, Levitt NS, Lambert EV, Collins MR, Morton NM, Andrew R, Walker BR & Seckl JR 2006 Glucocorticoid metabolism within superficial subcutaneous rather than visceral adipose tissue is associated with features of the metabolic syndrome. *Clinical Endocrinology* **65** 81–87.

Gustafsson J-A & Stenberg A 1974 Irreversible androgenic programming at birth of microsomal and soluble rat liver steroid metabolism by neonatal testosterone. *Journal of Biological Chemistry* **249** 711–718.

Hermanowski-Vosatka A, Gerhold D, Mundt SS, Loving VA, Lu M, Chen Y, Elbrecht A, Wu M, Doebber T, Kelly L *et al.* 2000 PPARalpha agonists reduce 11beta-hydroxysteroid dehydrogenase type 1 in the liver. *Biochemical and Biophysical Research Communications* **279** 330–336.

Hermanowski-Vosatka A, Balkovec JM, Cheng K, Chen HY, Hernandez M, Koo GC, Le Grand CB, Li Z, Metzger JM, Mundt SS *et al.* 2005 11β-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *Journal of Experimental Medicine* **202** 517–527.

Hoggard N, Mercer JG, Rayner DV, Moar K, Trayhurn P & Williams LM 1997 Localization of leptin receptor mRNA splice variants in murine peripheral tissues by RT-PCR and *in situ* hybridization. *Biochemical and Biophysical Research Communications* **232** 383–387.

Holmes MC, French KL & Seckl JR 1995 Modulation of serotonin and corticosteroid receptor gene expression in the rat hippocampus with circadian rhythm and stress. *Molecular Brain Research* **28** 186–192.

Horton R, Pasupuletti V & Antonipillai I 1993 Androgen induction of steroid 5α-reductase may be mediated via insulin-like growth factor-1. *Endocrinology* **133** 447–451.

Jamieson PM, Chapman KE, Edwards CRW & Seckl JR 1995 11β-Hydroxysteroid dehydrogenase is an exclusive 11β-reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology* **136** 4754–4761.

Jang C, Obeyesekere VR, Dilley RJ, Alford FP & Inder WJ 2006 11β-Hydroxysteroid dehydrogenase type 1 is expressed and is biologically active in human skeletal muscle. *Clinical Endocrinology* **65** 800–805.

Jansson JO, Ekberg S & Isaksson O 1985 Imprinting of growth hormone secretion, body growth, and hepatic steroid metabolism by neonatal testosterone. *Endocrinology* **117** 1881–1889.

Lee G-H, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI & Friedman JM 1996 Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379** 632–635.

Lindsay RS, Tataranni A, Permana P, Livingstone DEW, Wake DJ & Walker BR 2003 Subcutaneous adipose 11β-hydroxysteroid dehydrogenase type 1 activity and mRNA levels are associated with adiposity and insulinaemia in Pima Indians and Caucasians. *Journal of Clinical Endocrinology and Metabolism* **88** 2738–2744.

Liu Y, Nakagawa Y, Wang Y, Li R, Li X, Ohzeki T & Friedman TC 2003 Leptin activation of corticosterone production in hepatocytes may contribute to the reversal of obesity and hyperglycaemia in leptin deficient ob/ob mice. *Diabetes* **52** 1409–1416.

Liu Y, Nakagawa Y, Wang Y, Sakurai R, Tripathi PV, Lutfy K & Friedman TC 2005 Increased glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase type 1 expression in hepatocytes may contribute to the phenotype of type 2 diabetes in *db/db* mice. *Diabetes* **54** 32–40.

Livingstone DEW, Jones GC, Smith K, Andrew R, Kenyon CJ & Walker BR 2000 Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* **141** 560–563.

Livingstone DEW, McInnes KJ, Walker BR & Andrew R 2005 Increased A-ring reduction of glucocorticoids in obese rats: attenuation by insulin sensitisation. *Obesity Research* **13** 1523–1526.

Low SC, Moisan M-P, Edwards CRW & Seckl JR 1994 Glucocorticoids and chronic stress up-regulate 11b-hydroxysteroid dehydrogenase activity and gene expression in the hippocampus. *Journal of Neuroendocrinology* **6** 285–290.

Mahendroo MS, Cala KM & Russell DW 1996 5α-Reduced androgens play a key role in murine parturition. *Molecular Endocrinology* **10** 380–392.

Mahendroo MS, Cala KM, Hess DL & Russell DW 2001 Unexpected virilization in male mice lacking steroid 5α-reductase enzymes. *Endocrinology* **142** 4652–4662.

Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ & Seckl JR 2004 Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11beta-hydroxy-steroid dehydrogenase type 1-deficient mice. *Diabetes* **53** 931–938.

Morton NM, Densmore V, Wamil M, Ramage L, Nichol K, Bunger L, Seckl JR & Kenyon CJ 2005 A polygenic model of the Metabolic Syndrome with reduced circulating and intra-adipose glucocorticoid action. *Diabetes* **54** 3371–3378.

Nakano K, Inada Y, Masuzaki H, Tanaka T, Yasue S, Ishii T, Arai N, Ebihara K, Hosada K, Maruyama K *et al.* 2007 Bezafibrate regulated the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 in murine adipose tissue and 3T3-L1 adipocytes. *American Journal of Physiology. Endocrinology and Metabolism* **292** E1213–E1222.

Opperman UC, Netter KJ & Maser E 1995 Cloning and primary structure of murine 11β-hydroxysteroid microsomal carbonyl reductase. *European Journal of Biochemistry* **227** 202–208.

Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi MC & Grino M 2002 Expression of the mRNA coding for 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an *in situ* hybridisation study. *Journal of Clinical Endocrinology and Metabolism* **87** 2701–2705.

Phillips DIW, Walker BR, Reynolds RM, Flanagan DEH, Wood PJ, Osmond C, Barker DJ & Whorwood CB 2000 Low birthweight predicts elevated plasma cortisol concentrations in adults from three populations. *Journal of Internal Medicine* **35** 1301–1306.

Rask E, Olsson T, Söderberg S, Andrew R, Livingstone DEW, Johnson O & Walker BR 2001 Tissue-specific dysregulation of cortisol metabolism in human obesity. *Journal of Clinical Endocrinology and Metabolism* **86** 1418–1421.

Rask E, Walker BR, Söderberg S, Livingstone DEW, Eliasson M, Johnson O, Andrew R & Olsson T 2002 Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11β-hydroxysteroid dehydrogenase type 1 activity. *Journal of Clinical Endocrinology and Metabolism* **87** 3330–3336.

Raubenheimer PJ, Nyirenda MJ & Walker BR 2006 A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. *Diabetes* **55** 2015–2020.

Sahai M, Malladi P, Pan X, Paul R, Melin-Aldana H, Green RM & Whittington PF 2004 Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **287** G1035–G1043.

Da Silva BA, Bjorbaek C, Uotani S & Flier JS 1998 Functional properties of leptin receptor isoforms containing the Gln-Pro extracellular domain mutation of the fatty rat. *Endocrinology* **139** 3681–3690.

Surmi BK & Hasty AH 2008 Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. *Future Lipidology* **3** 545–556.

Tomlinson JW, Finney J, Hughes BA, Hughes S & Stewart PM 2008 Reduced glucocorticoid production rate, decreased 5α-reductase activity and adipose tissue insulin sensitization following weight loss. *Diabetes* **57** 1536–1543.

Tsilchorozidou T, Honour JW & Conway GS 2003 Altered cortisol metabolism in Polycystic Ovary Syndrome: insulin enhances 5α-reduction but not the elevated adrenal steroid production rates. *Journal of Clinical Endocrinology and Metabolism* **88** 5907–5913.

Voice MW, Seckl JR, Edwards CRW & Chapman KE 1996 11beta-Hydroxysteroid dehydrogenase type 1 expression in 2S FAZA hepatoma cells is hormonally regulated: a model system for the study of hepatic glucocorticoid metabolism. *Biochemical Journal* **317** 621–625.

Wake DJ, Rask E, Livingstone DEW, Soderberg S, Olsson T & Walker BR 2003 Local and systemic impact of transcriptional upregulation of 11β-hydroxysteroid dehydrogenase type 1 in human adipose tissues in obesity. *Journal of Clinical Endocrinology and Metabolism* **88** 3983–3988.

Wake DJ, Stimson RH, Tan GD, Andrew R, Homer NZM, Karpe F & Walker BR 2007*a* Influence of peroxisome proliferator-activated receptor (PPAR) α and γ agonists on 11β-hydroxysteroid dehydrogenase type 1 *in vivo* in humans. *Journal of Clinical Endocrinology and Metabolism* **92** 1848–1856.

Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DEW, Soderberg S, Andrew R, Olsson T, Yki-Jarvinen H & Walker BR 2007*b* The influence of intra-adipose enzymes generating estrogens and androgens on body fat distribution in idiopathic human obesity. *Clinical Endocrinology* **66** 440–446.

Walker BR & Andrew R 2006 Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. *Annals of the New York Academy of Sciences* **1083** 165–184.

Westerbacka J, Yki-Jarvinen H, Vehkavaara S, Häkkinen A-M, Andrew R, Wake DJ, Seckl JR & Walker BR 2003 Body fat distribution and cortisol metabolism in healthy men: enhanced 5β-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. *Journal of Clinical Endocrinology and Metabolism* **88** 4924–4931.

Whitaker EM, Shaw MA & Hervey GR 1983 Plasma oestradiol-17beta and testosterone concentrations as possible causes of the infertility of congenitally obese Zucker rats. *Journal of Endocrinology* **99** 485–490.

Zhao AZ, Shinohara MM, Huang D, Shimizu M, Eldar-Finkelman H, Krebs EG, Beavo JA & Bornfeldt KE 2000 Leptin induces insulin-like signalling that antagonizes cAMP elevation by glucagon in hepatocytes. *Journal of Biological Chemistry* **275** 11348–11354.

Zumoff B, Strain GW, Miller LK, Rosner W, Senie R & Seres D 1990 Plasma free and nonsex-hormone-binding-globulin-bound testosterone are decreased in obese men in proportion to their degree of obesity. *Journal of Clinical Endocrinology and Metabolism* **71** 929–931.

Received in final form 9 February 2009
Accepted 13 February 2009
Made available online as an Accepted Preprint
16 February 2009